

Short Communication

Influence of Exogenous Melatonin on Horizontal Transfer of *Escherichia coli* O157:H7 in Experimentally Infected Sheep

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Abstract

The objective of the current research was to determine if exogenous melatonin would exert a “protective” effect on the gastrointestinal tract of sheep and prevent or reduce the horizontal transfer of *Escherichia coli* O157:H7 from experimentally infected to noninfected or “naïve” sheep. Sixteen cross-bred ewes were housed indoors and adapted to a high concentrate ration. Ewes were randomly assigned to one of four rooms and treatment (three ewes/room, six ewes/treatment) and received either control (gelatin capsule only) or melatonin (5.0 mg/kg body weight [BW]/d). Four additional ewes served as “carrier” sheep (one/room) and were experimentally infected via oral gavage with *E. coli* O157:H7. Three days post-challenge, carrier ewes were housed with naïve sheep and remained with them for the remainder of the experimental period. Treatments were administered to the naïve sheep 1 day prior to introduction of the carrier sheep and on each of the remaining 7 days of the experimental period. Fecal samples were collected via rectal palpation from the carrier sheep daily throughout experiment and from the naïve sheep daily for 5 days, starting 2 days following introduction of the carriers. On day 8 of the experiment, all ewes were euthanized and tissues from the rumen, ileum, cecum, colon, and rectum as well as their respective lumen contents collected. The carrier sheep quickly infected the naïve ewes, which had similar fecal concentrations as the carrier animals throughout the 5-day sampling period. Melatonin treatment had no effect ($p > 0.10$) on daily fecal shedding, luminal content concentrations, or in the percentage of gastrointestinal tract tissue positive for the inoculated strain of *E. coli* O157:H7.

Introduction

ESCHERICHIA COLI O157:H7 is found primarily in ruminants (Capriola *et al.*, 2005) with fecal shedding typically more prevalent during the summer months (Chapman *et al.*, 1997; VanDonkersgoed *et al.*, 1999). We hypothesize that this seasonal variation is due to physiological responses within the animal in response to changing day-length (Edrington *et al.*, 2006). Previous research conducted in our laboratory with both naturally and experimentally infected cattle and sheep supports our hypothesis and indicated that the hormones melatonin, triiodothyronine, and thyroxine are likely involved (Edrington *et al.*, 2006, 2007, 2008; Schultz *et al.*, 2006).

Melatonin is especially intriguing given that secretion by the pineal gland is seasonal with serum concentrations

highest when *E. coli* O157:H7 prevalence is typically lowest (winter) and vice versa. The gastrointestinal tract (GIT) also produces melatonin, far exceeding pineal production (Bubenik, 2002). Given that certain segments of the GIT are preferred locations for *E. coli* O157:H7 in the ruminant, current research in our laboratory is investigating if GIT melatonin is secreted seasonally and if tissue concentrations can be correlated to fecal prevalence of *E. coli* O157:H7. Administration of exogenous melatonin to naturally colonized cattle (Edrington *et al.*, 2008) resulted in modest decreases in fecal shedding of *E. coli* O157:H7. Melatonin is reported to have a synergistic relationship with the immune system (Lissoni *et al.*, 1997; Drazen *et al.*, 2001) and GIT melatonin has been reported to play a protective role in the GIT (Bubenik 2002). Taken together with results of previous research, this suggests the

protective role of melatonin in the GIT may reduce the GIT colonization of *E. coli* O157:H7 and thereby influence fecal prevalence. The objective of the current research was to determine if exogenous melatonin would prevent the horizontal transfer of *E. coli* O157:H7 from experimentally infected to noninfected or "naïve" sheep.

Materials and Methods

Sixteen cross-bred (Suffolk × Rambouillet) ewes (avg. BW = 74 kg) were housed indoors in environmentally controlled facilities and randomly assigned to one of five isolation rooms. Over a 2-week period, ewes were adapted to an 80:20 concentrate to forage ration and adjusted to a 16-hour light and 8-hour dark photoperiod. Following the adaptation period, ewes from four of the five rooms ($n = 12$; 6 per treatment) were randomly assigned to treatment: control (gelatin capsule only) or melatonin (5.0 mg/kg BW/d). The ewes in the fifth room served as the "carrier" sheep and were experimentally infected via oral gavage with 10 mL of tryptic soy broth containing 3.5×10^9 colony-forming units (CFU)/mL *E. coli* O157:H7. Fecal samples were collected from the carrier sheep for two consecutive days to confirm shedding of the challenge strain as well as from the naïve sheep to culture for wild-type *E. coli* capable of growth on rifampicin-supplemented agar. On the third day post-challenge, one carrier ewe was introduced into each room of naïve sheep and remained in that room for the remainder of the experimental period. Treatments were administered to the naïve sheep 1 day prior to introduction of the carrier sheep and on each of the remaining 7 days of the experimental period. Thus each room contained three *E. coli* O157:H7 naïve and one carrier sheep though the course of the experimental period. Fecal samples were collected via rectal palpation from the carrier sheep daily throughout the experiment and from the naïve sheep daily for 5 days, starting 2 days following introduction of the carriers. On day 8 of the experiment, all ewes were humanely euthanized (Euthasol®, euthanasia solution; Delmarva Laboratories, Inc., Midlothian, VA) and tissues from the rumen, ileum, cecum, colon, and rectum as well as their respective lumen contents were aseptically collected for bacterial enumeration described below. Care, use, and handling of experimental animals was pre-approved by the Animal Care and Use Committee of the Food and Feed Safety Research Laboratory, U.S. Department of Agriculture.

Bacterial cultures and enumeration

The challenge strain of *E. coli* O157:H7 (strain 2336 obtained from the Field Disease Isolation Unit, Pullman, WA) was made resistant to rifampicin via successive cultivation in tryptic soy broth containing 25 µg/mL rifampicin. Fecal and luminal content samples (1 g) were homogenized and serially diluted (10-fold increments) in sterile phosphate-buffered saline, plated on MacConkey agar supplemented with 25 µg/mL rifampicin, and incubated (24 hours, 37°C) to enumerate *E. coli* O157:H7 concentrations. Tissue samples were incubated (24 hours, 37°C) in 20 mL GN Hajna with rifampicin, prior to plating and incubating as above for qualitative determination of the challenge strain.

Statistical analysis

Data were analyzed using SAS Version 8.02 (SAS Inst. Inc., Cary, NC). Data for quantitative fecal shedding and qualita-

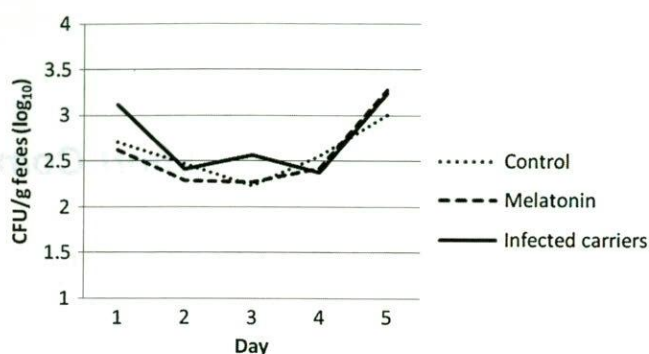


FIG. 1. Fecal shedding of *E. coli* O157:H7 (colony-forming units [CFU]/g feces log₁₀) in naïve sheep administered either 0 (control) or 5 mg melatonin/kg body weight (melatonin) and exposed to experimentally infected carrier sheep.

tive tissue and luminal content culture (positive or negative) were analyzed using the Proc Mixed and Proc Freq procedures (chi-square analysis), respectively. As treatments were individually administered and all sheep within a room were exposed to the carrier animal, individual sheep was used as the experimental unit in all statistical analyses.

Results

Prior to inoculation with *E. coli* O157:H7, sheep did not shed *E. coli* populations capable of growth on rifampicin-supplemented agar (data not shown). Fecal shedding of *E. coli* O157:H7 is presented in Fig. 1 by day, even though there was not a treatment × day interaction ($p > 0.10$) to illustrate the ability of the carrier sheep to quickly infect the naïve ewes. This resulted in similar fecal concentrations among the seeder and naïve animals throughout the 5-day sample collection period. Melatonin treatment had no effect ($p > 0.10$) on fecal shedding with concentrations similar to control animals. Populations of *E. coli* O157:H7 in luminal contents were lower in the rumen compared to other segments of the GIT, but not different ($p > 0.10$) among treatments (Table 1). Similarly, no treatment differences ($p > 0.10$) were observed in the percentage of GIT tissue positive for *E. coli* O157:H7 following enrichment. For each GIT segment, either five or six of the six tissue samples from the rumen, ileum, cecum, colon, and rectum in each treatment were culture positive (data not shown).

Discussion

Results of the current research highlight the ease of horizontal transmission of *E. coli* O157:H7 from infected to naïve animals when animals are housed in relatively confined spaces. The failure of our melatonin treatment to reduce or prevent horizontal transmission of *E. coli* O157:H7 could be a result of a number of factors. Our hypothesis, based on previous reports of melatonin's protective effects on the GIT (Lissoni *et al.*, 1997; Bubenik 2002), was that we could prevent colonization and subsequent shedding in naïve sheep with administration of exogenous melatonin. In hindsight, only 1 day of melatonin treatment prior to exposure to carrier animals was probably not sufficient time for the melatonin to exert a protective effect on the GIT. Considering the relatively

TABLE 1. POPULATIONS OF *E. COLI* O157:H7 FROM THE LUMINAL CONTENTS OF THE RUMEN, ILEUM, CECUM, COLON, AND RECTUM IN NAÏVE SHEEP ADMINISTERED EITHER 0 (CONTROL) OR 5 MG MELATONIN/KG BW (MELATONIN) AND EXPOSED TO EXPERIMENTALLY INFECTED CARRIER SHEEP

Item	Treatment		SEM	p > F
	Control	Melatonin		
Luminal contents (log ₁₀ CFU/g)				
Rumen	1.1	1.6	0.3	0.26
Ileum	3.7	3.4	0.39	0.63
Cecum	3.3	3.5	0.39	0.63
Colon	3.8	3.7	0.5	0.92
Rectum	3.1	3.7	0.48	0.4

BW, body weight; SEM, standard error of the mean; CFU, colony-forming units.

slow timeframe in which melatonin naturally responds to decreasing day-length, naïve ewes should have received the melatonin treatments for a longer time frame prior to *E. coli* O157:H7 exposure. Although, in previous research (Edrington *et al.*, 2008) we demonstrated an effect on fecal shedding in cattle during a 4-day dosing regimen of melatonin. Possibly the lack of effect observed in the current research was a result of melatonin degradation in the rumen with little melatonin reaching the lower GIT. However, based on previous research (Edrington *et al.*, 2008) in which we did observe an effect on fecal shedding of *E. coli* O157:H7 following dosing with an oral melatonin bolus, we hypothesized that the dose used in this experiment would be satisfactory and elicit similar effects.

The high concentration of *E. coli* O157:H7 shed by the seeder ewes (which would classify them as "super-shedders") could have overwhelmed any protective effects the melatonin may have had on the GIT. Exposure to seeder animals shedding lower concentrations might have produced a more "subtle" challenge to the naïve animals. Possibly the melatonin dose administered was not large enough to alter GIT melatonin concentrations. As we did not have a viable GIT melatonin assay at the time of the experiment we can only speculate. However, in previous research (Edrington *et al.*, 2008), melatonin administered to cattle at the same rate (5 mg/kg BW) was sufficient to influence fecal shedding of *E. coli* O157:H7 and we hypothesized that this dose would likewise be sufficient to influence horizontal transfer of this pathogen.

In evaluating which hormones (known to respond to changing day-length) to examine, melatonin based on the secretion patterns that are inverse to *E. coli* O157:H7 shedding patterns, stands out as a logical choice. However, a number of hormones offer intriguing possibilities, some of which we have demonstrated can also influence fecal shedding of *E. coli* O157:H7 (Edrington *et al.*, 2007). The most probable scenario likely involves multiple hormones, or other compounds yet to

be discovered, functioning in a cascade of events to influence *E. coli* O157:H7 populations.

Acknowledgment

Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Disclosure Statement

No competing financial interests exist.

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